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# **NEXTflex™ Rapid DNA-Seq Kit (1 ng – 1 µg)** **(Illumina Compatible)**

**Catalog #: 5144-01 (8 reactions)**

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The NEXTflex™ Rapid DNA-Seq Kit is intended for research use only. NEXTflex is a trademark of Bioo Scientific Corporation.



## GENERAL INFORMATION

### Product Overview

The NEXTflex™ Rapid-DNA Seq Kit is designed for 2 hour DNA library construction with as little as 1 ng – 1 µg of fragmented DNA. The kit can be used to prepare single, paired-end and multiplexed genomic DNA libraries for sequencing using Illumina® GAllx, MiSeq and HiSeq platforms. The NEXTflex™ 1-step End-Repair and Adenylation simplifies workflow and shortens hands-on library construction time. A bead-based, gel-free size selection protocol eliminates the need for agarose gel size selection. In addition, the availability of up to 96 unique adapter barcodes facilitates high-throughput applications.

There are five main steps involved in preparing genomic DNA for sequencing: DNA extraction, DNA fragmentation, DNA end repair / adenylation, adapter ligation and PCR amplification. The NEXTflex™ Rapid DNA-Seq Kit contains the necessary material to take the user's purified and fragmented genomic DNA through preparation and amplification for loading onto flow cells for sequencing.

### Contents, Storage and Shelf Life

The NEXTflex™ Rapid DNA-Seq Kit contains enough material to prepare 8 genomic DNA samples for Illumina® compatible sequencing. The shelf life of all reagents is 12 months when stored properly. All components can be safely stored at -20°C.

Kit Contents	Amount
<b>CLEAR CAP</b>	
NEXTflex™ End-Repair & Adenylation Buffer Mix	120 µL
NEXTflex™ End-Repair & Adenylation Enzyme Mix	24 µL
<b>PURPLE CAP</b>	
NEXTflex™ Ligase Enzyme Mix	380 µL
NEXTflex™ DNA-Seq Adapter 1	20 µL
<b>GREEN CAP</b>	
NEXTflex™ PCR Master Mix	96 µL
NEXTflex™ Primer Mix	16 µL
<b>WHITE CAP</b>	
Nuclease-free Water	1 mL
NEXTflex™ Resuspension buffer	(2)1 mL
<b>RED CAP</b>	
NEXTflex™ Sizing Solution	1.8 mL



## **Required Materials Not Provided**

- 1 ng - 1 µg of fragmented genomic DNA in up to 40 µL nuclease-free water.
- NEXTflex™ DNA Barcodes – 6 / 12 / 24 / 48 (Cat # 514101, 514102, 514103, 514104) or NEXTflex-96™ DNA Barcodes (Cat # 514106) or NEXTflex™ ChIP-Seq Barcodes – 6 / 12 / 24 / 48 (Cat # 514120, 514121, 514122, 514123) or NEXTflex-96™ ChIP-Seq Barcodes (Cat # 514124)
- Ethanol 100% (room temperature)
- Ethanol 80% (room temperature)
- AIR™ DNA Fragmentation Kit (Bioo Scientific, Cat # 5135-01) or Covaris System (S2, E210)
- 96 well PCR Plate Non-skirted (Phenix Research, Cat # MPS-499) or similar
- 96 well Library Storage and Pooling Plate (Fisher Scientific, Cat # AB-0765) or similar
- Adhesive PCR Plate Seal (BioRad, Cat # MSB1001)
- Agencourt AMPure XP 5 mL (Beckman Coulter Genomics, Cat # A63880)
- Magnetic Stand -96 (Ambion, Cat # AM10027) / or / similar
- Thermocycler
- 2, 10, 20, 200 and 1000 µL pipettes / multichannel pipettes
- Nuclease-free barrier pipette tips
- Vortex



## **Warnings and Precautions**

Bioo Scientific strongly recommends that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, you may contact your local distributor, or contact Bioo Scientific at [nextgen@biooscientific.com](mailto:nextgen@biooscientific.com).

- Do not use the kit past the expiration date.
- DTT in buffers may precipitate after freezing. If precipitate is seen, vortex buffer for 1-2 minutes or until the precipitate is in solution. The performance of the buffer is not affected once the precipitate is in solution.
- Ensure pipettes are properly calibrated as library preparations are highly sensitive to pipetting error.
- Do not heat the DNA Adapter above room temperature.
- Try to maintain a laboratory temperature of 20°–25°C (68°–77°F).
- DNA sample quality may vary between preparations. It is the user's responsibility to utilize high quality DNA. DNA that is heavily nicked or damaged may cause library preparation failure. Absorbance measurements at 260 nm are commonly used to quantify DNA and 260 nm / 280 nm ratios of 1.8 - 2.0 usually indicate relatively pure DNA. Other quantification methods using fluorescent dyes may also be used. The user should be aware that contaminating RNA, nucleotides and single-stranded DNA may affect the amount of usable DNA in a sample preparation.
- DNA fragmentation methods that physically break up DNA into pieces of less than 800 bp are compatible with this kit. These methods include the AIR™ DNA Fragmentation Kit (5135-01), based on the nebulization of DNA, or acoustic technologies that fragment DNA in a controlled and accurate manner. We do not recommend enzymatic methods of fragmentation as this may introduce sequence bias into the preparation.
- It is highly recommended that NEXTflex™ Primer Mix be used during PCR amplification. Inadvertent use of an incorrect primer sequence can potentially result in elimination of the index.

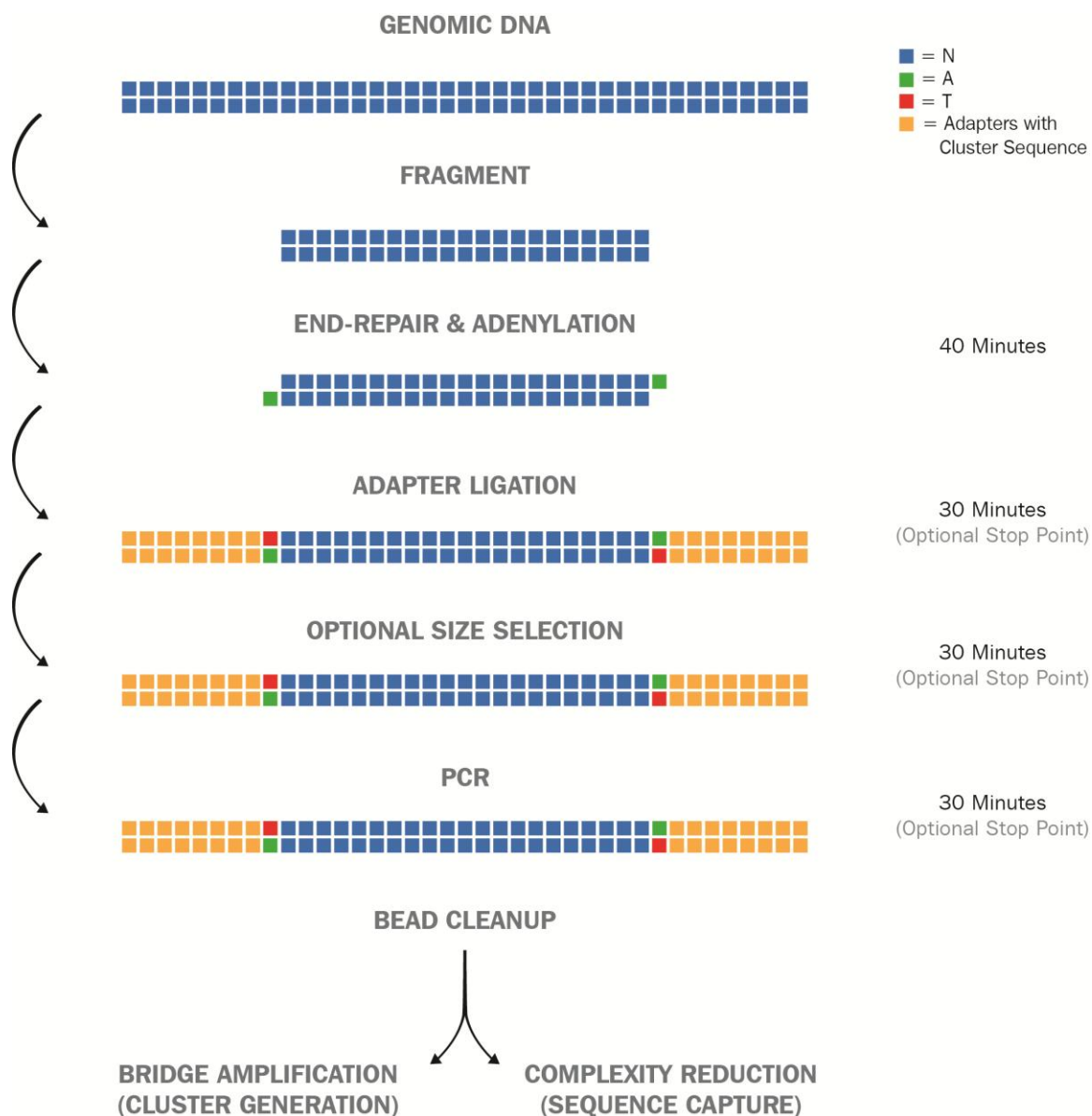
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# NEXTflex™ RAPID DNA SAMPLE PREPARATION PROTOCOL

## NEXTflex™ Rapid DNA Sample Preparation Flow Chart

**Figure 1:**

Sample flow chart with approximate times necessary for each step.





## **Starting Material**

The NEXTflex™ Rapid DNA-Seq Kit has been optimized and validated using genomic DNA. Starting with 1 ng - 1 µg of high quality fragmented genomic DNA will allow you to perform at least 8 reactions per adapter or barcoded adapter (see page 3, Warnings and Precautions).

## **Reagent Preparation**

1. Briefly spin down each component to ensure material has not lodged in the cap or side of tube. Keep on ice and vortex each NEXTflex™ Mix just prior to use.
2. DTT in buffers may precipitate after freezing. If precipitate is seen in any mix, vortex for 1 minute or until the precipitate is in solution. The performance of the mix is not affected once the precipitate is in solution.
3. Allow Agencourt AMPure XP Beads to come to room temperature and vortex the beads until homogenous.

## **STEP A: End-Repair & Adenylation**

### **Materials**

#### *Bioo Scientific Supplied*

##### **CLEAR CAP**

NEXTflex™ End-Repair & Adenylation Buffer Mix

NEXTflex™ End-Repair & Adenylation Enzyme Mix

##### **WHITE CAP**

Nuclease-free Water

#### *User Supplied*

Fragmented DNA in 32 µL (or less) nuclease-free water

96 well PCR Plate

Adhesive PCR Plate Seal

Agencourt AMPure XP Magnetic Beads

Microcentrifuge

Ice

1. For each sample, combine the following reagents on ice in a nuclease-free 96 well PCR Plate:

_ µL	Nuclease-free Water
_ µL	Fragmented DNA (1 ng - 1 µg)
15 µL	NEXTflex™ End-Repair & Adenylation Buffer Mix
3 µL	NEXTflex™ End-Repair & Adenylation Enzyme Mix
<hr/>	
50 µL	TOTAL
2. Apply adhesive PCR plate seal and incubate on a thermocycler for 20 minutes at 25°C followed by 20 minutes at 72°C (**DO NOT CYCLE**).



## STEP B: 3' Adapter Ligation

### Materials

*Bioo Scientific Supplied*

**PURPLE CAP**

NEXTflex™ Ligation Mix and

**WHITE CAP**

Nuclease-free Water

*User Supplied*

Thermocycler (set to 37°C)

**50 µL of End Repaired DNA (from STEP A)**

NEXTflex™ DNA Barcodes – 6 / 12 / 24 / 48 (Cat # 514101, 514102, 514103, 514104) or

NEXTflex-96™ DNA Barcodes (Cat # 514106) or NEXTflex™ ChIP-Seq Barcodes – 6 / 12 / 24 /

48 (Cat # 514120, 514121, 514122, 514123) or NEXTflex-96™ ChIP-Seq Barcodes (Cat #

514124)

\*The table below indicates appropriate adapter concentrations required for various starting material amounts. If starting with 1 ng - 100 ng of input DNA, dilute adapter with Nuclease-free Water accordingly to the table. If starting with 250 ng - 1 µg of input DNA, adapter dilution is not required.

Input DNA	Barcodes Used	Desired Adapter Concentration	Adapter Dilution Required
1 ng	ChIP-Seq (0.6 µM)	0.6 µM	None
10 ng	ChIP-Seq (0.6 µM)	0.6 µM	None
100 ng	DNA-Seq (25 µM)	3 µM	1 : 8.3
250 ng	DNA-Seq (25 µM)	25 µM	None
500 ng	DNA-Seq (25 µM)	25 µM	None
1 µg	DNA-Seq (25 µM)	25 µM	None

1. Combine the following in the PCR plate:

50 µL End Repaired DNA (from Step A)

47.5 µL NEXTflex™ Ligation Mix

2.5 µL NEXTflex™ DNA Barcode or NEXTflex™ ChIP-Seq Barcode

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100 µL TOTAL

2. Apply adhesive PCR plate seal and incubate on a thermocycler for 15 minutes at 22°C.





## STEP C: Optional Bead Size Selection

### Materials

*Bioo Scientific Supplied*

#### **CLEAR CAP BOTTLE**

NEXTflex™ Sizing Solution

NEXTflex™ Resuspension buffer

*User Supplied*

Agencourt AMPure XP Magnetic Beads (room temperature)

80% Ethanol, freshly prepared (room temperature)

Magnetic Stand

**100 µL of Adapter Ligated DNA (from STEP B)**

If you wish to perform bead size selection, follow the entire protocol in Step C. If not performing size selection, only follow steps 1-15 then steps 27-32, using 37.5 µL of Sizing Solution during step 9. Size Selection may not be optimal for inputs ≤ 5 ng.

Use the following table for bead size selection:

	300 – 400 bp:	350 - 500 bp:	400 – 600 bp:	500 – 700 bp:	650 – 800 bp:
<b>Lower Cutoff</b>	37.5 µL	35 µL	32.5µL	30 µL	28.5 µL
<b>Upper Cutoff</b>	35 µL	32.5 µL	30 µL	28.5 µL	27 µL

Ensure all reagents are at room temperature. Vortex AMPure XP and Sizing Solution thoroughly prior to use. Use a fresh dilution of 80% ethanol during wash steps.

1. Add 60 µL of AMPure XP Beads to each sample and gently pipette the entire volume up and down 10 times.
2. Incubate sample at room temperature for 5 minutes.
3. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes or until the supernatant appears completely clear.
4. Remove and discard clear supernatant taking care not to disturb beads. Some liquid may remain in wells.
5. With plate on stand, gently add 200 µL of freshly prepared 80% ethanol to each magnetic bead pellet and incubate plate at room temperature for 30 seconds. Carefully, remove ethanol by pipette.
6. Repeat step 5, for a total of 2 ethanol washes. Ensure all ethanol has been removed.
7. Remove the plate from the magnetic stand and let dry at room temperature for 2 minutes.
8. Resuspend dried beads with 50 µL Resuspension Buffer. Gently pipette entire volume up and down 10 times mixing thoroughly. Ensure beads are no longer attached to the side of the well.

9. Add **Lower Cutoff** volume of Sizing Solution to the resuspended bead pellet as in the table below for the desired final library size distribution. For example, if the desired selection range is 350 – 500 bp, add 35 µL of Sizing Solution to the 50 µL resuspended bead pellet. Mix thoroughly.

Selection Range	300 – 400 bp:	350 - 500 bp:	400 – 600 bp:	500 – 700 bp:	650 – 800 bp:
Lower Cutoff	37.5 µL	35 µL	32.5 µL	30 µL	28.5 µL

10. Incubate sample at room temperature for 5 minutes.
11. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes or until the supernatant appears completely clear.
12. Remove and discard clear supernatant taking care not to disturb beads. Some liquid may remain in wells.
13. With plate on stand, gently add 200 µL of freshly prepared 80% ethanol to each magnetic bead pellet and incubate plate at room temperature for 30 seconds. Carefully remove ethanol by pipette.
14. Repeat step 13, for a total of 2 ethanol washes. Ensure all ethanol has been removed.
15. Remove the plate from the magnetic stand and let dry at room temperature for 2 minutes.
16. Resuspend dried beads with 50 µL Resuspension Buffer. Gently, pipette entire volume up and down 10 times mixing thoroughly. Ensure beads are no longer attached to the side of the well.
17. Add **Upper Cutoff** volume of Sizing Solution to the resuspended bead pellet as in the table below for the desired final library size distribution. For example, if the desired selection range is 350 – 500 bp, add 32.5 µL of Sizing Solution to the 50 µL resuspended bead pellet. Mix thoroughly.

Selection Range	300 – 400 bp:	350 - 500 bp:	400 – 600 bp:	500 – 700 bp:	650 – 800 bp:
Upper Cutoff	35 µL	32.5 µL	30 µL	28.5 µL	27 µL

18. Incubate sample at room temperature for 5 minutes.
19. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes or until the supernatant appears completely clear.
20. **Do not discard the sample in this step.** Transfer the **Sample Volume** of clear sample to a new well as in the table below for the desired final library size distribution.  
**Be careful not to disrupt the magnetic bead pellet or transfer any magnetic beads with the sample.** This is your size selected DNA.

Selection Range	300 – 400 bp:	350 - 500 bp:	400 – 600 bp:	500 – 700 bp:	650 – 800 bp:
Sample Volume	83 µL	80 µL	77 µL	75 µL	72 µL

21. Add **Bead Volume** of AMPure XP Beads to each well containing sample as in the table below for the desired final library size distribution. Gently pipette the entire volume up and down 10 times and incubate at room temperature for 5 minutes.

Selection Range	300 – 400 bp:	350 - 500 bp:	400 – 600 bp:	500 – 700 bp:	650 – 800 bp:
Bead Volume	50 µL	48 µL	46 µL	45 µL	43 µL



22. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes or until the supernatant appears completely clear.
23. Gently remove and discard the clear supernatant, taking care not to disturb beads. Some liquid may remain in wells.
24. With the plate on the stand, gently add 200  $\mu$ L of freshly prepared 80% ethanol to each magnetic bead pellet and incubate plate at room temperature for 30 seconds. Carefully remove ethanol by pipette.
25. Repeat step 24, for a total of 2 ethanol washes. Ensure all ethanol has been removed.
26. Remove the plate from the magnetic stand and let dry at room temperature for 3 minutes.
27. Resuspend dried beads with 21  $\mu$ L Resuspension Buffer. Gently pipette entire volume up and down 10 times, mixing thoroughly. Ensure beads are no longer attached to the side of the well.
28. Incubate resuspended beads at room temperature for 2 minutes.
29. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes or until the sample appears completely clear.
30. Gently transfer 20  $\mu$ L of clear sample to a new well.
31. If you wish to pause your experiment, the procedure may be safely stopped at this step with samples stored at -20°C. To restart, thaw frozen samples on ice before proceeding.
32. Proceed to Step D.



## STEP D: PCR Amplification

### Materials

*Bioo Scientific Supplied*

**GREEN CAP**

NEXTflex™ PCR Master Mix

**CLEAR CAP BOTTLE**

NEXTflex™ Resuspension buffer

*User Supplied*

Thermocycler

96 Well PCR Plate

NEXTflex™ Primer Mix

Agencourt AMPure XP Magnetic Beads (room temperature)

80% Ethanol, freshly prepared (room temperature)

Magnetic Stand

**\*20 µL of Adapter Ligated DNA (from STEP C)**

\*The following table lists recommended PCR cycles:

Input DNA (ng)	Ligated DNA (µL)	PCR cycles
1	20	15
10	5	13 - 15
100	5	10 - 12
250	5	8 - 10
500	5	6 - 8
1000	5	6 - 8

1. For each sample, combine the following reagents on ice in the PCR plate.

_µL	Ligated DNA
_µL	Nuclease-free H <sub>2</sub> O
12 µL	NEXTflex™ PCR Master Mix
2 µL	NEXTflex™ Primer Mix
<hr/>	
50 µL	TOTAL

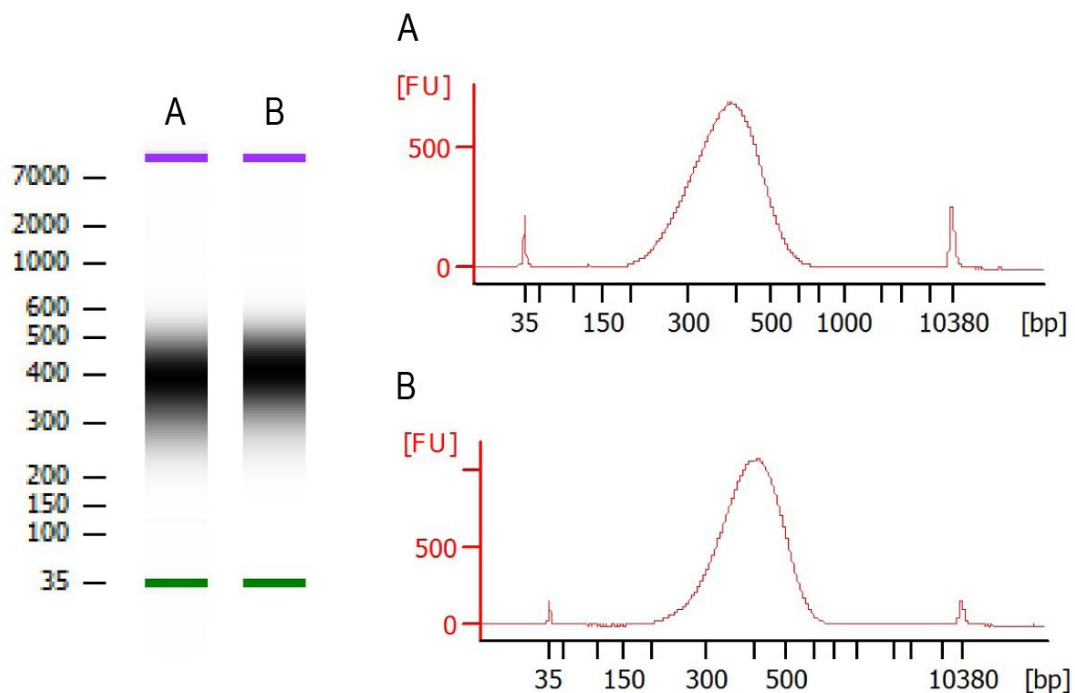
2. Apply adhesive PCR plate seal and place in thermocycler for the following PCR cycles:

2 min	98°C	Repeat 6 -15 cycles*
30 sec	98°C	
30 sec	65°C	
60 sec	72°C	
4 min	72°C	



3. Add 30  $\mu$ L of AMPure XP Beads to each sample and gently pipette the entire volume up and down 10 times.
4. Incubate at room temperature for 5 minutes.
5. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes or until the supernatant appears completely clear.
6. Remove and discard clear supernatant taking care not to disturb beads. Some liquid may remain in wells.
7. With plate on stand, gently add 200  $\mu$ L of freshly prepared 80% ethanol to each magnetic bead pellet and incubate plate at room temperature for 30 seconds. Carefully remove ethanol by pipette.
8. Repeat step 7, for a total of 2 ethanol washes, and ensure all ethanol has been removed.
9. Remove plate from magnetic stand and let dry at room temperature for 2 minutes.
10. Resuspend dried beads with 21  $\mu$ L Resuspension Buffer. Gently pipette entire volume up and down 10 times, mixing thoroughly. Ensure beads are no longer attached to the side of the well.
11. Incubate resuspended beads at room temperature for 2 minutes.
12. Place the 96 well PCR Plate on the magnetic stand at room temperature for 5 minutes or until the supernatant appears completely clear.
13. Gently transfer 20  $\mu$ L of clear supernatant to a well of a new 96 well PCR Plate.
14. Examine your library by gel or Agilent Bioanalyzer.
15. qPCR is recommended to quantify DNA library templates for optimal cluster density. This can be performed using any qPCR quantification kit with the NEXTflex™ Primer Mix.

## LIBRARY VALIDATION



### High Sensitivity DNA Chip Ladder / Electropherogram

A) 1 ng input NEXTflex™ 15 cycle PCR product.

B) 1 µg input NEXTflex™ 6 cycle PCR product.



## APPENDIX A

### *Oligonucleotide Sequences*

NEXTflex™	Sequence
DNA Adapter	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCGATGTATCTCGTATGCCGTCTTCTGCTTG
Primer 1	5'AATGATACGGCGACCACCGAGATCTACAC
Primer 2	5'CAAGCAGAAGACGGCATACGAGAT

**RELATED PRODUCTS*****Illumina Compatible DNA NGS Kits and Adapters***

Product	Catalog Number
NEXTflex™ 16S V4 Amplicon-Seq kit – 12	4201-02
NEXTflex™ 16S V4 Amplicon-Seq kit – 24	4201-03
NEXTflex™ 16S V4 Amplicon-Seq kit – 48	4201-04
NEXTflex™ 16S V4 Amplicon-Seq kit – 288	4201-05
NEXTflex™ DNA Barcodes – 6	514101
NEXTflex™ DNA Barcodes – 12	514102
NEXTflex™ DNA Barcodes – 24	514103
NEXTflex™ DNA Barcodes – 48	514104
NEXTflex-96™ DNA Barcodes	514106
NEXTflex™ DNA Sequencing Kit (8 reactions)	5140-01
NEXTflex™ DNA Sequencing Kit (48 reactions)	5140-02
NEXTflex™ Rapid DNA Sequencing Kit (8 reactions)	5144-01
NEXTflex™ Rapid DNA Sequencing Kit (48 reactions)	5144-02
NEXTflex™ Bisulfite-Seq kit (8 reactions)	5119-01
NEXTflex™ Bisulfite-Seq kit (48 reactions)	5119-02
NEXTflex™ Bisulfite-Seq Barcodes – 6	511911
NEXTflex™ Bisulfite-Seq Barcodes – 12	511912
NEXTflex™ Msp 1 (8 reactions)	511921
NEXTflex™ Msp 1 (48 reactions)	511922
NEXTflex™ Pre-Capture Combo Kit -6	5140-51
NEXTflex™ Pre-Capture Combo Kit -12	5140-52
NEXTflex™ Pre-Capture Combo Kit -24	5140-53
NEXTflex™ Pre-Capture Combo Kit -96	5140-54
NEXTflex™ DNA Barcode Blockers - 6 for SeqCap v3	514131
NEXTflex™ DNA Barcode Blockers - 12 for SeqCap v3	514132
NEXTflex™ DNA Barcode Blockers - 24 for SeqCap v3	514133
NEXTflex-96™ DNA Barcode Blockers - 96 for SeqCap v3	514134
NEXTflex™ ChIP-Seq Kit (8 reactions)	5143-01
NEXTflex™ ChIP-Seq Kit (48 reactions)	5143-02
NEXTflex™ ChIP-Seq Barcodes – 6	514120
NEXTflex™ ChIP-Seq Barcodes – 12	514121
NEXTflex™ ChIP-Seq Barcodes – 24	514122
NEXTflex™ ChIP-Seq Barcodes – 48	514123
NEXTflex-96™ ChIP-Seq Barcodes	514124
NEXTflex™ PCR-Free DNA Sequencing Kit (8 reactions)	5142-01
NEXTflex™ PCR-Free DNA Sequencing Kit (48 reactions)	5142-02
NEXTflex™ PCR-Free Barcodes – 6	514110
NEXTflex™ PCR-Free Barcodes – 12	514111
NEXTflex™ PCR-Free Barcodes – 24	514112
NEXTflex™ PCR-Free Barcodes – 48	514113
NEXTflex™ Methyl-Seq 1 kit (8 reactions)	5118-01
NEXTflex™ Methyl-Seq 1 kit (48 reactions)	5118-02



***DNA Fragmentation***

Product	Catalog Number
AIR™ DNA Fragmentation Kit (10 reactions)	5135-01
AIR™ DNA Fragmentation Kit (40 reactions)	5135-02

***Illumina Compatible RNA NGS Kits and Adapters***

Product	Catalog Number
NEXTflex™ RNA-Seq Kit (8 reactions)	5129-01
NEXTflex™ RNA-Seq Kit (48 reactions)	5129-02
NEXTflex™ RNA-Seq Barcodes – 6	512911
NEXTflex™ RNA-Seq Barcodes – 12	512912
NEXTflex™ RNA-Seq Barcodes – 24	512913
NEXTflex™ RNA-Seq Barcodes – 48	512914
NEXTflex-96™ RNA-Seq Barcodes	512916
NEXTflex™ Directional RNA-Seq Kit (dUTP-Based) (8 reactions)	5129-05
NEXTflex™ Directional RNA-Seq Kit (dUTP-Based) (48 reactions)	5129-06
NEXTflex™ Directional RNA-Seq Barcodes – Set A	513311
NEXTflex™ Directional RNA-Seq Barcodes – Set B	513312
NEXTflex™ Directional RNA-Seq Barcodes – Set C	513313
NEXTflex™ Directional RNA-Seq Barcodes – Set D	513314
NEXTflex™ Small RNA Sequencing Kit (24 reactions)	5132-01
NEXTflex™ Small RNA Sequencing Kit (48 reactions)	5132-02
NEXTflex™ Small RNA Barcodes – Set A	513301
NEXTflex™ Small RNA Barcodes – Set B	513302
NEXTflex™ Small RNA Barcodes – Set C	513303
NEXTflex™ Small RNA Barcodes – Set D	513304

Bioo Scientific also offers library prep kits and barcodes for the Ion Torrent, 5500 SOLiD and SOLiD 4 sequencing platforms. For more information about any of these kits visit our website at [www.biooscientific.com](http://www.biooscientific.com).



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